Concentrates of Fat-Soluble Constituents of Leaf Meal Extracts

PREPARATION BY MOLECULAR DISTILLATION

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Fat-soluble constituents of vegetable leaf extracts may be concentrated and partially separated by molecular distillation. Prior to distillation, it is necessary to remove phospholipides by saponification or acetone precipitation, and to dissolve the residual material in a suitable carrier oil. By distilling at 80° to 220° C. and 1 to 10 microns pressure, a series of concentrates containing phytol, tocopherol, sterols, carotene, and xanthophyll is obtained.

PREVIOUS publications from this laboratory have shown that properly prepared vegetable leaf meals (*2*) are excellent sources of carotene (*12*), xanthophyll (*15*), chlorophyll (*15*), tocopherol (*13*), and sterols (*14*). In addition, vitamin *K* (*3*) and many other less well known compounds may be present. The conventional methods for isolating or concentrating any individual compound from leaves are usually so specific that many of the other products are destroyed or ignored (*3, 4, 10, 15*). For more complete utilization of vegetable leaves, a method suitable for concentrating or partially separating a number of leaf lipides is desirable.

A literature survey showed that the process known as short-path or molecular distillation had been successfully applied to the concentration of heat-sensitive substances such as vitamins A and D from marine oils (*5, 6*) and tocopherols from vegetable oils (*8*). This method has been brought to a high theoretical and technological stage in this country largely through the research of Hickman and co-workers. The purpose of this paper is to present the methods developed in this laboratory for the preparation of leaf extracts suitable for molecular distillation and to discuss some of the distillation products.

The leaf meals were prepared from broccoli, Lima bean, rhubarb, and spinach leaf wastes. They were dried to approximately 5% residual moisture and freed of stems by a process developed at this laboratory (*2*). The dry meal was then ground in a cutter over a 1/16-inch screen. Commercially dehydrated alfalfa leaf meal was used for comparison.

Twenty to twenty-five pound batches of leaf meal were extracted with acetone or hexane in a large Soxhlet apparatus (*15*) for 12 hours. The extracts thus obtained constituted the crude leaf lipide extract. The components of this mixture which were quantitatively studied and the references to the analytical methods used are as follows: carotene (*12*), xanthophyll (*15*), tocopherol (*13*), and sterol (*14*). In addition, chlorophyll, phytol (derived from chlorophyll), and phospholipides were investigated to a limited extent.

A cyclic, falling-film type of molecular still with a capacity of one liter was secured from Distillation Products, Inc. Similar models have been described in considerable detail by Hickman (*6, 7*).

Two distinct processes were involved in the preparation of fat-soluble concentrates: (*a*) the preparation of a crude extract in a form suitable for molecular distillation in the falling-film still and (*b*) the subsequent distillation of the extract. Figure 1 is a flow sheet of the various processes.

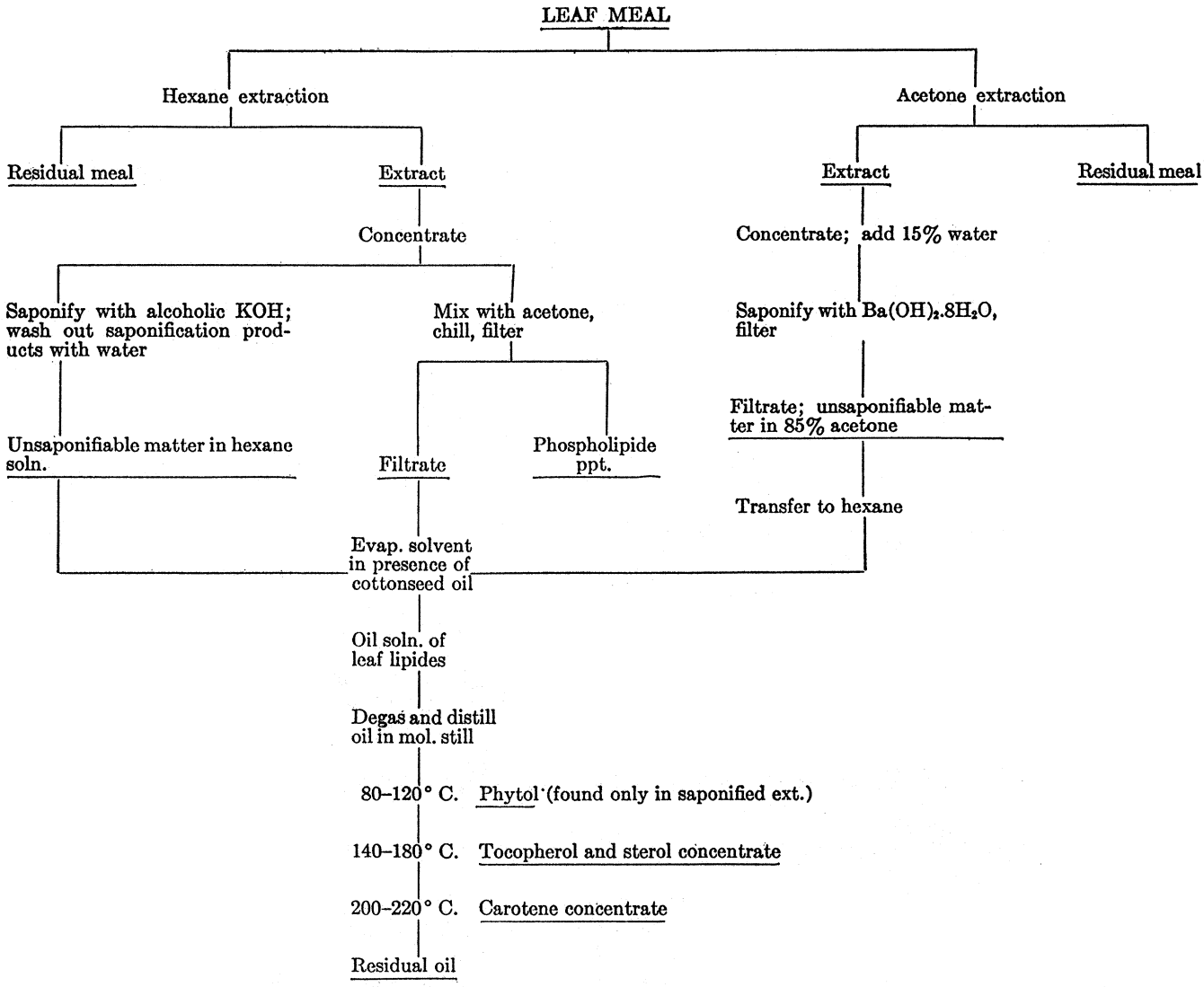

Figure 1. Flow Sheet of Processes Involved in Preparing Fat-Soluble Concentrates from Vegetable Leaf Meals

PREPARATION OF EXTRACTS

PRELIMINARY EXPERIMENTS. The products to be distilled in the falling-film still should be free-flowing liquids with low vapor-pressure at temperatures of 50° to 80° C. (*6*, *7*). The residues obtained by evaporating all the solvent from acetone or hexane leaf extracts were semisolid at 50° to 80° C. and difficult to free of solvent. It was obvious that they could not be distilled in the apparatus at hand. To obtain a free-flowing, solvent-free solution, it was necessary to dissolve the lipides in a vehicle such as cottonseed oil; approximately 5 parts of oil by weight to 1 part of lipide was used. Attempts to distill such solutions invariably failed at the degassing stage owing to violent and prolonged splashing and foaming, which was believed to be due to phospholipides. After considerable experimentation several procedures were devised which removed phospholipides and thus made possible successful molecular distillation of leaf lipides dissolved in carrier oils.

SAPONIFICATION METHODS. Acetone solutions of leaf lipides were saponified with barium hydroxide by the method of Petering *et al.* (*10*). After the barium cake was filtered and washed, the filtrate containing the unsaponifiable fraction was transferred to hexane and dried over anhydrous sodium sulfate. A quantity of U.S.P. cottonseed oil equal in weight to the total solids present was dissolved in the hexane solution, and the solvent was evaporated under vacuum and mild heat. The resultant oil solutions were easily degassed and distilled.

Hexane leaf extracts were saponified for 30 minutes with 10% potassium hydroxide in ethanol as in the method of Wall *et al.* (*15*) except that the saponification was conducted at room temperature. After the saponification products were washed out with water, the hexane solutions were dried with anhydrous sodium sulfate and the solvent was evaporated in the presence of cottonseed oil as described above. Although the cold saponification of the hexane leaf extracts did not remove a large proportion of the total solids, the more oil-insoluble substances and phospholipides were apparently removed. The remaining lipides could be dissolved in an equal weight of oil, and the resultant solutions were easily degassed and distilled.

Table I shows the effect of saponification on the composition of some typical acetone and hexane leaf extracts. Use of barium hydroxide for saponification resulted in loss of carotene and sterol, and alcoholic potassium hydroxide caused loss of tocopherol. In addition, both methods destroyed the saponifiable fraction.

ACETONE PRECIPITATION METHOD. When unsaponified acetone leaf extracts were concentrated and chilled, voluminous precipitates formed. These could be easily filtered without filter aid and the filtrate contained almost all the desired constituents. Th cottonseed oil solutions prepared from such extracts were rather viscous and extremely difficult to degas. Since hexane extracts less nonlipide material from leaves than acetone, the above procedure was applied to hexane extracts as follows: The extract from 20 to 25 pounds of leaf meal was concentrated in vacuum, with mild heating, almost to dryness. Residue was mixed with 8 to 10 liters of acetone, gently refluxed for a few minutes, and the allowed to stand overnight at 4° C. The voluminous precipitat which formed was filtered on a large Büchner funnel and sucked a dry as possible but not washed. Cottonseed oil was added to the filtrate (1 part oil to 1 part solids), and the solvent evaporated. The resultant oil solutions were free flowing at room temperature, and were successfully degassed and distilled. Since the oil solu-

TABLE I. COMPOSITION OF ACETONE AND HEXANE EXTRACTS[a] OF LEAF MEALS BEFORE AND AFTER SAPONIFICATION (IN GRAMS)

Compound	Acetone Ext. Before	Acetone Ext. After	Hexane Ext. Before	Hexane Ext. After
Broccoli Leaf Meal				
Carotene	3.3	1.9	4.3	4.2
Xanthophyll	3.7	3.2	3.6	3.4
Chlorophyll	50.0	Trace	30.0	4.3
Sterol	11.7	6.2	11.7	11.7
Tocopherol	2.5	2.3	3.6	2.0
Total solids	600.0	74.0	380.0	260.0
Rhubarb Leaf Meal				
Carotene	2.6	2.0	3.0	3.0
Xanthophyll	2.2	2.0	2.5	2.4
Chlorophyll[b]	50.0	2.8	22.6	4.35
Sterol	10.0	6.2	8.2	8.0
Tocopherol	5.7	4.8	3.8	2.3
Total solids	680.0	100.0	278.0	184.0

[a] All extracts were made from 20 pounds (9 kg.) of leaf meal. The acetone and hexane extracts were produced from different batches of meal.
[b] The chlorophyll in dehydrated rhubarb leaves is converted almost entirely to pheophytin.

tions were somewhat more viscous than those produced by saponification, the rate of degassing was also slower.

Table II shows the composition of hexane leaf extracts before and after acetone precipitation. It is apparent that, although a considerable quantity of solids was removed by acetone precipitation, almost all the desired constituents were present in the filtrate.

TABLE II. COMPOSITION OF HEXANE EXTRACTS OF LEAF MEALS BEFORE AND AFTER ACETONE PRECIPITATION[a] (IN GRAMS)

Compound	Broccoli Before	Broccoli After	Rhubarb Before	Rhubarb After	Lima Bean Before	Lima Bean After	Spinach Before	Spinach After	Alfalfa Before	Alfalfa After
Carotene	4.5	4.4	2.6	2.6	2.0	2.0	2.1	2.2	1.1	1.0
Xanthophyll	2.3	2.2	1.5	1.3	...	...	...	...	...	...
Chlorophyll	22.0	19.2	22.6	20.0	22.0	20.6	12.6	10.8	10.9	10.9
Tocopherol	3.5	3.5	5.9	5.6	6.3	6.4	2.1	2.1	1.3	1.2
Sterol	10.8	10.1	6.7	6.5	6.5	6.5	5.6	5.3	6.7	6.2
Total solids	412.0	202.0	219.0	144.0	271.0	209.0	179.0	104.0	262.0	145.0

[a] All extracts were made from 20 pounds (9 kg.) of leaf meal.

The acetone precipitate has not been thoroughly investigated. The data in Table III show that it consisted chiefly of saponifiable products and contained phospholipides. Future studies may show that these are useful components. The acetone precipitation procedure has considerable advantage over the saponification methods. It involves fewer and less complicated steps, and none of the components of the leaf extract are destroyed. It is the preferred procedure at this laboratory for investigating the total leaf lipide fraction.

TABLE III. COMPOSITION OF CRUDE ACETONE PRECIPITATE FROM HEXANE EXTRACTS OF LEAF MEALS (IN PER CENT)

Leaf Meal	Phosphorus	Nitrogen	Choline	Saponifiable Matter
Broccoli	0.16	0.51	0.77	67.5
Rhubarb	0.18	0.80	1.97	89.2
Lima bean	0.14	0.76	0.62	91.6
Spinach	0.21	0.72	1.40	85.6
Alfalfa	0.15	0.76	1.27	88.0

MOLECULAR DISTILLATION

METHODS. The technique of distillation in a falling-film still similar to the author's was described in detail by Hickman (7). The methods used by the author were similar and necessitate only a brief description:

The oil solutions of the leaf lipides were warmed to 50° C. and degassed in the molecular still at a temperature not exceeding 50° to 60° C. and a pressure of 100 to 150 microns (Pirani gage). When a quiet distilland was obtained (usually after two cycles over the evaporator), the pressure was reduced to 10 microns or less, and the evaporator temperature raised until distillation started. In most cases the distilland was cycled twice at the initial distillation temperature, and then the temperature was raised 20° C. and the process repeated. In this manner a temperature range from 80° to 220° C. was studied for the various leaf lipides in a cottonseed oil vehicle. The distillates obtained at each temperature were weighed and analyzed.

The distillates were, for the most part, viscous and small in quantity as compared with the distilland; at times it was necessary to warm the sides of the condenser with a microburner to collect the viscous distillate in the receiver. As a result the various fractions collected tended to overlap. To determine more accurately the possibility of separating the various components, analytical distillations with constant yield oil (1) were carried out in a manner similar to that described by Hickman (7) with 20° C. temperature increments and 10-minute cycle periods. The two methods gave comparable results for the substances studied.

UNSAPONIFIED AND SAPONIFIED LEAF EXTRACTS. Under the experimental conditions the unsaponified oil solutions prepared from various leaf meals by the acetone precipitation method began to distill between 120° and 140° C. The fractions collected between 120° and 180° were orange, viscous products which solidified at room temperature.

Since the cottonseed oil vehicle alone did not distill to any appreciable extent below 180° C., it was apparent that the fractions collected up to 180° were of leaf origin. Analysis showed that they contained most of the tocopherols and sterols originally present.

Above 180° C. the cottonseed oil began to distill. The distillates collected between 180° and 220° were deep red oils. They contained carotene and xanthophyll, but were low in tocopherol and sterols. Chlorophyll did not distill in the temperature range investigated.

In contrast to the unsaponified extracts, the saponified leaf lipides in cottonseed oil began to distill between 80° and 100° C. The fraction collected at these temperatures was a yellow oil which consisted mainly of phytol. The phytol was obtained by saponification of chlorophyll. The fractions collected at higher temperatures were similar in composition and appearance to those obtained from unsaponified extracts.

Table IV presents the distillation data of an unsaponified and a saponified broccoli leaf extract, respectively, in cottonseed oil. Table V shows the data obtained by distillation of an unsaponified broccoli extract mixed with constant yield oil. The effect of increments in distillation temperature on the concentration of the various compounds studied was similar in all cases. Maximum concentration of tocopherol and sterol was found at 160° C., and maximum carotene and xanthophyll concentration at 200° C. Hickman reports comparable results for sterols and tocopherols isolated from marine and vegetable oils (8). Similar results were obtained with extracts from different leaf sources.

RELATION BETWEEN MOLECULAR WEIGHT AND SEPARATION OF LEAF CONSTITUENTS. From the previous discussion, it is apparent that certain groups of compounds in leaf extracts can be reasonably well separated by simple molecular distillation in the falling-film still. Phytol (found only in saponified extracts) can be largely separated from tocopherols and sterols, which in turn can be separated from carotene and xanthophyll, and the latter separated from chlorophyll. On the other hand, tocopherol cannot be separated from sterol, nor can carotene be separated from xanthophyll. The degree of separation was closely correlated with molecular weight and fitted in well with the theory developed in Hickman's discussion of this subject (5, 6). Table VI shows the interrelation between molecular weight and distillation temperature. It is apparent that, in order to separate

TABLE IV. DISTILLATION OF UNSAPONIFIED AND SAPONIFIED BROCCOLI LEAF MEAL EXTRACTS

Product	Appearance	Weight, Grams	Carotene %	Carotene Total grams	Xanthophyll %	Xanthophyll Total grams	Tocopherol %	Tocopherol Total grams	Sterol %	Sterol Total grams
20 Pounds Leaf Meal, Acetone Precipitation										
Original oil soln. of leaf lipides	Green oil	202 lipide + 200 oil	1.10	4.42	0.55	2.20	0.87	3.50	2.52	10.10
Distillate at 100° C.	Mist on condenser	...	...	...	...	...	...	...	...	...
120° C.	Yellow wax	3.9	Trace		Trace		1.31	0.05	6.4	0.25
140° C.	Yellow wax	8.7	Trace		Trace		8.35	0.73	23.0	2.00
160° C.	Orange wax	11.0	Trace		Trace		10.00	1.10	35.7	3.93
180° C.	Red wax	7.3	1.37	0.10	0.69	0.05	7.55	0.55	27.9	2.04
200–220° C.	Red oil	46.1	3.00	1.38	0.78	0.36	1.45	0.67	4.48	2.00
Residue	Green oil	300.0		0.98	0.31	0.93	0.24	0.20	0.0	0.0
Total (including original oil)		377.0[a]		2.46		1.34		3.30		10.22
20 Pounds Leaf Meal, Ba(OH)₂ Saponification										
Original oil soln. of leaf lipides	Green oil	70.5 lipide + 200.0 oil	0.65	1.8	...	...	0.74	2.0	2.6	6.0
Distillate at 100° C.	Pale yellow oil	22.0[b]	...	...	...	...	Trace		Trace	
120° C.	Mist on condenser	...	...	...	...	...	...	...	...	...
140° C.	Orange wax	5.0	Trace		...	...	8.0	0.40	26.8	1.34
160–180° C.	Orange wax	6.0	1.50	0.09	...	...	10.0	0.60	33.2	1.99
200–220° C.	Red oil	57.7	1.35	0.78	...	...	1.0	0.57	2.14	1.24
Residue	Red oil	170.0	0.05	0.09	...	...	0.06	0.10	0.30	0.50
Total (including original oil)		260.7		0.96				1.67		5.07

[a] It was impossible to drain all the residual oil from the still; this largely accounts for the difference in weight between the original oil and the distilled fractions and residue.
[b] 80 to 90% phytol.

leaf constituents by molecular distillation in the falling-film still, there must be a considerable difference in molecular weight.

Since most of the known saponifiable leaf compounds are of high molecular weight and most of the unsaponifiable constituents are of relatively low molecular weight, molecular distillation presents an excellent method for separating the unsaponifiable fraction without recourse to the often destructive action of alkali. This is strongly confirmed by the fact that the 140° to 180° C. fractions distilled from saponified and unsaponified extracts have almost the same concentration of tocopherol and sterol (Table IV).

VEHICLE OILS. The U.S.P. cottonseed oil, which was used as a vehicle for the leaf lipides throughout this investigation, was useful not only because it acted as a solvent for the lipides but also because of its distillation behavior. Under our experimental conditions, little oil distilled below 200° C.[1] Therefore leaf fractions distilling below this temperature were obtained practically free of vehicle oil. Most of the cottonseed oil distilled between 200° and 240° C. The carotene which also distilled in this range was thus obtained in a bland, edible oil solution. Edible oils with similar properties, such as corn, soybean, or wheat germ, could be used in a similar manner.

Saturated oils such as coconut or palm oils were not suitable because a considerable fraction distilled below 200° C. Attempts to use various petroleum oil fractions as vehicles for the leaf lipides were unsuccessful because a considerable portion of the leaf fraction was immiscible in the petroleum oils even at elevated temperatures.

RECOVERY AND CONCENTRATION OF DISTILLED COMPOUNDS. The chief objective of molecular distillation is to concentrate a desired compound with as little loss as possible. The distillates from various leaf extracts were therefore prepared in order to determine the recovery and concentration of tocopherol, sterol, and carotene. The recovery data are given in Table VII and the concentration results in Table VIII. All the data were obtained from unsaponified extracts prepared from 20 pounds of each of the various leaf meals.

The recovery of tocopherol, sterol, and carotene from the leaf extracts was remarkably uniform. The over-all retention of tocopherol and sterol was at least 90%, and in most cases it was higher. The total recovery of carotene was only about 50%, and similar values were found for xanthophyll. In the most potent fractions, 61 to 68% of tocopherol, 63 to 74% of sterol, and 29 to 32% of carotene were recovered. It is apparent that distillation in the falling-film still causes considerable destruction of the carotenoid fraction and may also cause isomerization of the carotenes (*17*). This is primarily a time-temperature effect, for when the temperature did not exceed 170° to 180° C. and the number of cycles were reduced, about 70% of the carotene was recovered[2]. Hickman (*6*) also reported a 50% loss of carotene with the falling-film still but made the important observation that 95% of the carotene was recovered in a centrifugal still. It is probable, therefore, that distillation of carotene from vegetable leaf extracts in centrifugal stills would result in much higher recovery.

The data on the concentration of tocopherol, sterol, and carotene from cottonseed oil–leaf lipide solutions reveal at first glance

[1] The U.S.P. cottonseed oil used throughout these investigations contained about 0.1 gram of tocopherol and 0.2 gram of sterol in the average 200-gram charge. Since these quantities were very low in comparison with those in the leaf extracts, stripping tocopherol and sterol from the oil prior to distillation was not considered necessary.

[2] It is significant that both the distilled and residual carotene had the same effect as vitamin A ester on growth and feed efficiency of young chicks when each was mixed with mash to give levels of 500, 1500, and 3000 I.U. per pound (*11*).

TABLE V. DISTILLATION OF UNSAPONIFIED BROCCOLI LEAF MEAL EXTRACT IN CONSTANT YIELD OIL

Distillate at	Weight of Fraction, Grams	Carotene, %	Tocopherol, %	Sterol, %
100° C.	10.1	0.00	0.06	0.00
120° C.	12.2	0.00	0.35	1.90
140° C.	12.7	0.00	0.86	2.72
160° C.	11.7	0.03	1.45	5.25
180° C.	14.5	0.12	1.32	4.54
200° C.	14.8	0.66	0.86	2.66
220° C.	17.0	0.59	0.10	0.64

TABLE VI. RELATION BETWEEN MOLECULAR WEIGHT AND DISTILLATION TEMPERATURE OF SOME LEAF LIPIDES

Compound	Mol. Weight	Distn. Temp. at Max. Concn., ° C.	Distn. Range[a], ° C.	$\sqrt{M/T}$[b]
Phytol	296.5	100	80–120	0.89
Sterol	414	160	140–180	0.98
Tocopherol	430.7	160	140–180	0.99
Carotene	536.9	200	180–220	1.07
Xanthophyll	566.9	200	180–220	1.09
Chlorophyll	902.5	Does not distill[c]	...	...

[a] Small quantities of these compounds are always found above and below the given distillation range.
[b] According to Hickman (*6*), $\sqrt{M/T}$ approximates unity, where M = molecular weight and T = distillation temperature, ° K.
[c] Chlorophyll does not distill up to 220° C., the maximum temperature investigated.

TABLE VII. EFFECT OF MOLECULAR DISTILLATION ON RECOVERY OF CAROTENE, STEROL, AND TOCOPHEROL FROM UNSAPONIFIED LEAF MEAL EXTRACTS (IN PER CENT)

	Tocopherol Recovered		Sterol Recovered		Carotene Recovered	
Leaf Meal	140–180° C.	All other fractions	140–180° C.	All other fractions	200–220° C.	Residue
Alfalfa	61	32	68	31	31	19
Broccoli	68	26	74	21	31	22
Lima bean	65	30	68	29	30	21
Rhubarb	66	31	66	28	32	16
Spinach	64	30	63	27	29	6

a less uniform picture than the recovery results. Invariably the concentrations of these compounds in the most potent distillates were proportional to the concentrations present in the original oil solutions. Since these varied greatly, depending on the leaf source, the proportions present in the most potent distillates also showed large variations. On the other hand, the increase in concentration of these compounds was uniform for all the leaf extracts studied. The concentration of tocopherol and sterol was increased approximately tenfold and that of carotene, twofold. The carotene picture was complicated by the fact that half the carotene was destroyed and that the oil used as a vehicle distilled at the same temperature.

Depending on the source, the tocopherol concentrations ranged from 3 to 21%, sterol from 12 to 36%, and carotene from 0.5 to 3%. Lima bean and rhubarb leaf meals were the best tocopherol sources, and broccoli leaf meal was the best source for carotene and sterols.

PURIFICATION OF DISTILLATES. No attempt was made to increase the potency of the fractions by redistillation or partial reflux. From Hickman's account of the fractionation obtainable with centrifugal stills (*9*), it is conceivable that higher concentrations of these compounds could be obtained in large-scale industrial distillations. However, the various substances concentrated by molecular distillation in the falling-film still could often be purified by conventional methods, which were not particularly successful when applied to the original crude leaf extracts. This phase of the work is still in progress, but the following brief account will show the scope of the method.

TABLE VIII. EFFECT OF MOLECULAR DISTILLATION ON CONCENTRATION OF CAROTENE, STEROL, AND TOCOPHEROL FROM UNSAPONIFIED LEAF MEAL EXTRACTS (IN PER CENT)

	Tocopherol			Sterol			Carotene	
	Oil soln. of leaf	Dist. at 140–180° C.		Oil soln. of leaf	Dist. at 140–180° C.		Oil soln. of leaf	Dist. at 200–220° C., av.
Leaf Meal	lipides	Av.	Max.	lipides	Av.	Max.	lipides	
Alfalfa	0.39	3.2	...	2.40	30.0	...	0.32	0.50
Broccoli	0.87	8.8	10.0	2.52	29.6	35.7	1.10	3.00
Lima bean	1.75	19.9	20.7	1.65	16.2	21.6	0.45	1.06
Rhubarb	1.62	17.0	20.0	1.89	23.6	27.0	0.76	1.50
Spinach	0.70	10.0	...	1.75	15.5	...	0.70	1.1

PHYTOL. Oil solutions prepared from acetone extracts saponified with barium hydroxide were molecularly distilled, and the oily fraction was collected at 80° to 120° C. and redistilled from a small Claisen flask under 1-mm. pressure. The majority of the oil (80 to 90%) distilled at 155–157° C. Its carbon and hydrogen values proved that the redistilled oil was practically pure phytol (carbon, 81.46, theory, 81.06; hydrogen, 14.08, theory, 13.60). The iodine number was 90.75, which agrees well with the values 90.5 to 91.2 given by Willstätter and Stoll (*16*).

STEROL-TOCOPHEROL FRACTION. Distillates collected at 140° to 180° C. in the molecular still were considerably purified by fractional crystallization from hot ethanol. In this manner sterols and other compounds insoluble in cold ethanol were separated from tocopherol. In preliminary experiments crystalline sterols (probably mixtures) which contained little or no nonsterol components were isolated. In addition some crystalline nonsterol components were separated. At the same time the tocopherol concentration in the noncrystalline residue was increased two to three times.

CAROTENE. The carotene concentration can be increased by saponifying the cottonseed oil vehicle which distills with the carotene at 200° to 220° C. The unsaponifiable residue contains 10 to 20% carotene.

SUMMARY

Crude hexane or acetone leaf meal extracts could not be molecularly distilled without preliminary treatment to remove phospholipides and oil-insoluble constituents. This treatment consisted in saponifyng hexane or acetone solutions or concentrating hexane extracts, and then precipitating the phospholipides with acetone. The latter treatment was preferable because none of the leaf constituents were destroyed. In either case the solvent was then evaporated in the presence of vegetable oil, such as cottonseed; equal parts by weight of oil and total leaf solids were used.

The solvent-free oil solution was then transferred to a falling-film molecular still, and the oil was degassed and finally distilled at pressures of 10 microns or less over a temperature range of 80° to 220° C. The following distillate fractions were obtained: phytol (from saponified extracts only) at 80° to 120° C., tocopherol-sterol fraction at 140° to 180° C., and a carotene–xanthophyll–cottonseed oil distillate at 200° to 220° C.

The over-all recovery of tocopherol and sterol was 90% or more, but approximately half the carotene and xanthophyll was destroyed. About 60 to 70% of the tocopherol and sterol and about 30% of the carotene were found in the most potent fractions. The concentration of tocopherol and sterol was increased tenfold over the concentration originally present in the oil–leaf lipide solution. The concentration of carotene was increased only twofold, owing to the fact that half of it was destroyed and that the cottonseed oil vehicle distilled with the carotene.

Preliminary experiments indicate that the distilled fractions can be considerably purified by conventional methods. Pure phytol was prepared by redistillation of the crude concentrate. Crystalline sterol and nonsterol compounds were isolated by fractional crystallization of the tocopherol-sterol distillate from ethanol. The carotene concentration can be increased to 10 to 20% by saponifying the cottonseed oil vehicle.

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